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# Investigation of miR-222 as a potential biomarker in diagnosis of patients with methamphetamine abuse disorder

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## Abstract

**Background:** Methamphetamine abuse disorder is an important social and health problem worldwide. Diagnosis and confirmation of patients with methamphetamine abuse using serum are important in many fields. MicroRNAs (miRNAs) are small non-coding oligonucleotides and recently suggested as a biomarker for earlier diagnosis of several human disorders. Therefore, in this study, we investigated miR-222 and miR-212 expressions in blood of patients with methamphetamine abuse disorder comparison with healthy control subjects.

**Results:** The results revealed that the expression of blood miR-222 is significantly increased (12.9-fold change) in patients with methamphetamine abuse disorders compared to healthy controls ( $p < 0.05$ ). However, expression of miR-212 is at the same levels in both patients and healthy controls ( $p > 0.05$ ).

**Conclusions:** In general, we suggested that the miR-222 may play a potentially important role in pathogenesis of methamphetamine abuse disorder and can be considered as an applied tool for identifying individuals with methamphetamine abuse disorder.

**Keywords:** Addiction, Methamphetamine, MicroRNA-222, MicroRNA-212, Biomarker

## Background

In recent years, methamphetamine addiction has been suggested as a chronic debilitating cerebral disease which is one of the costliest diseases in the current society [1]. In the year 2012, approximately 34,000,000 persons have used methamphetamine at some time in their lives in the worldwide [1]. Methamphetamine is a highly addictive psychostimulant that is readily synthesized with relatively low-cost materials, making the drug inexpensive and readily available [2]. So far, a limited study has been conducted on the molecular mechanisms of methamphetamine abuse disorder; thus, there are no effective pharmaceutical therapies for patients with methamphetamine abuse disorder. Therefore, more studies are

required to the development of effective diagnosis and treatment approaches for methamphetamine abuse disorder [3].

The noninvasive and rapid diagnosis methods of methamphetamine abuse using peripheral blood samples are important. Currently, diagnosis of methamphetamine abuse is mainly conducted by liquid chromatography (LC)-mass spectrometry (MS) or gas chromatography (GC)-mass spectrometry (MS) methods through detection of the methamphetamine metabolites in the peripheral blood, serum or plasma of patients [4]. However, these methods require special materials and equipment as well as are time-consuming. So, development of blood biomarkers is still in great need to quickly diagnosis of methamphetamine abuses or other drugs addiction.

MicroRNAs (miRNAs), small non-coding oligonucleotides, are involved regulation of various genes expression during brain development and differentiation [5]. Moreover, a high miRNAs diversity is observed in mature

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neurons, with approximately 100 different miRNAs involved in development and differentiation human neurons. Evidence suggested that the expression of the several miRNAs in the brain may be involved in memory formation, neuronal morphogenesis, and drug addiction [6, 7]. Previous studies provided that miRNAs are involved in the pathogenesis of drugs addiction, such as alcohol, nicotine, cocaine, and other drugs [8, 9].

Various molecular targets have been described for miR-222 and miR-212 in the human brain, such as synaptic transmission, inflammation, development of neurological mediators, and angiogenesis [10, 11]. However, there is no study on molecular mechanisms of miR-222 and miR-212 expression and function in Iranian patients with methamphetamine abuse disorder. Therefore, we evaluated expression of miR-222 and miR-212 in peripheral blood of Iranian patients with methamphetamine abuse disorder.

## Methods

### Study subjects

This case-control study consisted of 120 subjects (20–40 years old) referred to educational hospitals of Tabriz, Iran, from 2018 to 2019. The case group composed of 60 patients with methamphetamine abuse disorder, newly diagnosed without treatment. The patients with addiction to other drugs except methamphetamine were excluded from this study. The control group composed 60 healthy subjects, referred to health check-up and routine examination. All patients and healthy controls with chronic disease, major psychiatric disorders, brain disease, and cardiovascular disease were excluded. Moreover, all patients and healthy controls were recruited from the population of East Azerbaijan province of Iran, age- and ethnically matched. The clinical characteristics, demographic information, and lifestyle (age, gender, marital status, literacy levels, drug use history, and syphilis infection status) were collected through interviews and questionnaires.

### RNA extraction

5 mL peripheral blood samples were received from all patients and healthy controls after 12 h of fasting. The obtained blood samples were collected into ethylene diamine tetra acetic acid (EDTA) containing vials as an anticoagulant. The total RNA extraction was performed using an RNA extraction kit (GeneAll Biotechnology, Germany) according to instructions of the manufacturer's instructions. The quantity of the extracted RNA samples was evaluated by a nanodrop instrument. Moreover, the quality of extracted RNA samples was evaluated by electrophoresis on 1% agarose gel. The extracted RNA

samples were stored at  $-20\text{ }^{\circ}\text{C}$  until molecular analysis [12].

### cDNA synthesis

For this purpose, polyadenylation of RNA samples was performed using Poly-A polymerase enzyme in  $37\text{ }^{\circ}\text{C}$  (30 min) and next  $65\text{ }^{\circ}\text{C}$  (20 min). The synthesis of cDNA was conducted using BON-RT adaptor primers as the following condition:  $16\text{ }^{\circ}\text{C}$  for 30 min,  $42\text{ }^{\circ}\text{C}$  for 30 min,  $85\text{ }^{\circ}\text{C}$  for 5 min, and store at  $4\text{ }^{\circ}\text{C}$  [12].

### Quantitative real-time PCR

A TaqMan probe-based RT-qPCR assay was conducted to evaluate miR-222 and miR-212 expression in the blood samples of all patients and healthy controls in triplicate. The total volume (15  $\mu\text{L}$ ) consisted cDNA (1.5  $\mu\text{L}$ ), PCR buffer (7.5  $\mu\text{L}$ ), each primer (0.5  $\mu\text{L}$ ), and diethyl pyrocarbonate (DEPC) RNase free water (5  $\mu\text{L}$ ). Expression of miR-222 and miR-212 was performed as the following condition: 1 cycle in  $94\text{ }^{\circ}\text{C}$  for 1 min (initial denaturation), 45 cycles in  $94\text{ }^{\circ}\text{C}$  for 10 s (denaturation), 45 cycles in  $94\text{ }^{\circ}\text{C}$  for 30 s (annealing), and 45 cycles in  $72\text{ }^{\circ}\text{C}$  for 20 s (extension). The used primers were Has- miR-222-F-ACTACATCTGGCTACTGG and Has- miR-212-F-GGTAACAGTCTCCAGTCA. The threshold cycle (CT) was determined, and relative expressions of miR-222 and miR-212 were normalized to U6 and calculated using the  $2^{-\Delta\text{Cq}}$  method [13].

### Statistical analysis

For statistical analysis of the obtained data, we used SPSS (version 21.0) and GraphPad Prism 6 software. The clinical characteristics and miRNAs expression are presented as mean  $\pm$  SEM or mean  $\pm$  SD. Difference of miRNAs expression between case and healthy controls was investigated by Pearson's correlation analysis. Moreover, difference of clinical characteristics between case and healthy controls was investigated by Chi-square and independent sample t-test. The difference with a  $p < 0.05$  was considered statistically significant [13].

## Results

### Participant characteristics

The statistical analysis of age, marital status, and syphilis infection showed a significant difference between patients with methamphetamine abuse disorder and healthy controls ( $p < 0.05$ ), whereas BMI and educational degree between patients with methamphetamine abuse disorder and healthy controls were similar and without significant difference ( $p > 0.05$ ). The clinical characteristics, demographic information, and lifestyle of the studied case and control groups are presented in Table 1.

**Table 1** The clinical characteristics, demographic information, and lifestyle of the studied patients with methamphetamine abuse disorder and healthy controls

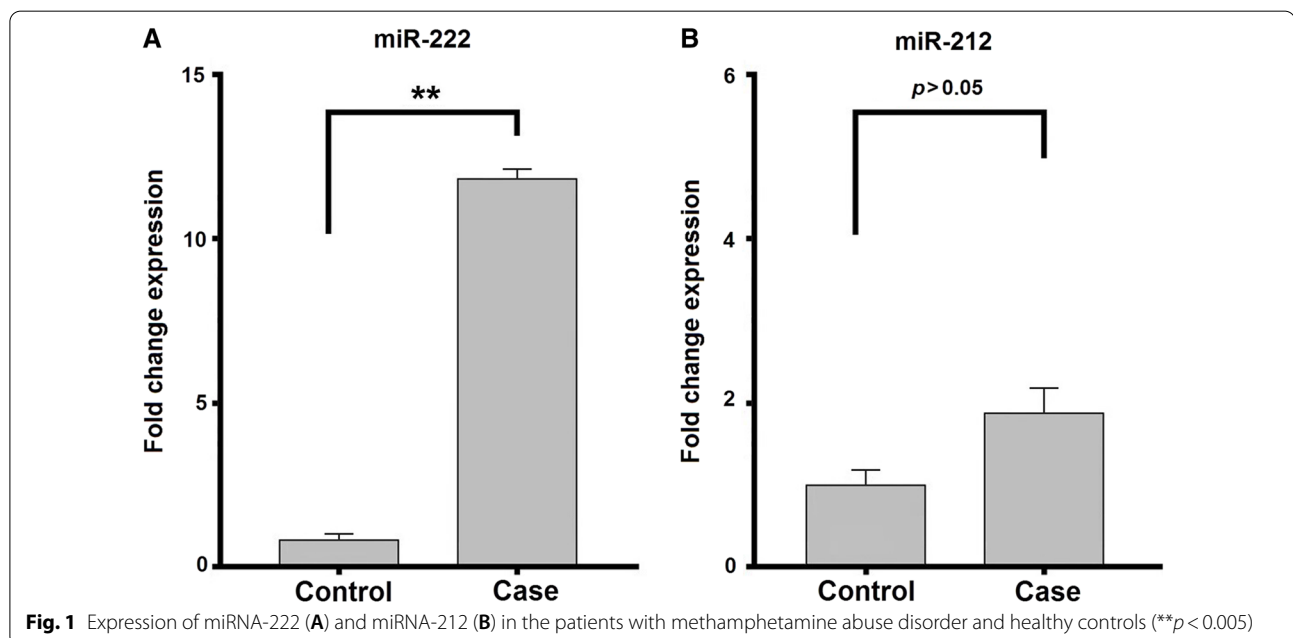
Variable	Case (n = 60)	Controls (n = 60)	p-value
Age, years	28.41 ± 2.51	30.73 ± 9.22	0.100
BMI, kg/m <sup>2</sup>	22.19 ± 2.18	22.34 ± 2.55	0.529
<i>Marital status</i>			
Married, no (%)	28 (46.6%)	42 (70.0%)	0.008
Single, no (%)	18 (30.0%)	12 (20.2%)	
Divorced, no (%)	14 (23.3%)	6 (10.0%)	
<i>Educational degree</i>			
Under diploma and diploma, no (%)	44 (73.3%)	36 (60.0%)	0.265
Higher diploma, no (%)	16 (26.6%)	24 (40.0%)	
<i>Drug use history</i>			
Onset age of drug use (years)	24.78 ± 2.28	–	–
Drug use time (years)	4.56 ± 3.24	–	–
Times of addicts drug per day	1.87 ± 2.11	–	–
<i>Drug manner</i>			
Injection, no (%)	4 (6.6%)	–	–
Oral inhalation, no (%)	56 (93.3%)	–	–
<i>Syphilis infection status</i>			
Positive, no (%)	8 (13.3%)	0 (0.0%)	< 0.001
Negative, no (%)	52 (86.6%)	60 (100.0%)	

BMI: body mass index; data are presented as mean ± SD; the  $p < 0.05$  is statistically significant

### Expression of miRNAs

Our study revealed that the expression of miR-222 was significantly increased (12.9-fold change) in patients with methamphetamine abuse disorder than in healthy controls ( $p < 0.05$ ). However, miR-212 expression was

increased insignificantly in patients with methamphetamine abuse disorder than healthy controls ( $p > 0.05$ ). Expression of miR-222 and miR-212 in the case and control groups is presented in Fig. 1.



### Diagnostic potential of miRNAs

The analysis of receiver operating characteristics (ROC) curve was used to evaluate the diagnostic potential of miR-222 and miR-212 for the patients with methamphetamine abusers disorder. The ROC curve analysis of miR-222 showed that the area under the curve (AUC) score was 0.898 to discriminate patients with methamphetamine abuse disorder from healthy controls (Fig. 2A). Moreover, this analysis for miR-212 showed AUC score of 0.694 to discriminate patients from healthy control (Fig. 2B). These results demonstrated that miR-222 could be an appropriate biomarker for diagnosing of patients with methamphetamine abuse disorder.

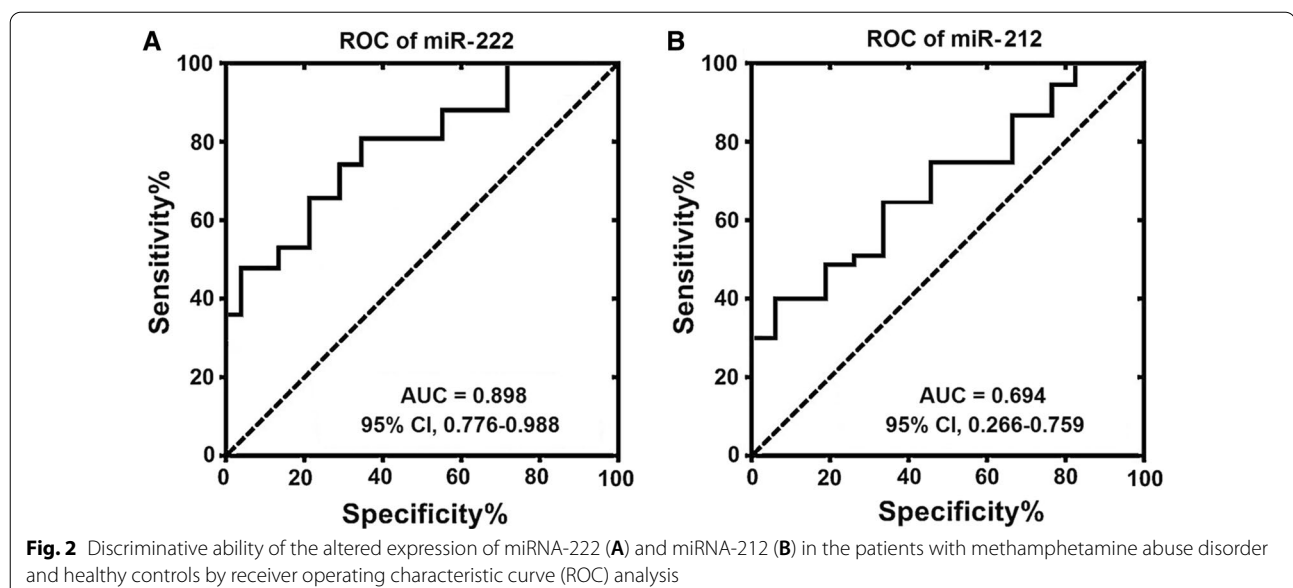
### Discussion

Previous studies reported that dysregulated various miRNAs are involved in neurogenesis, neuronal function, and other neurobiological processes, such as synaptic plasticity, which are associated with drug addiction [12–14]. Moreover, brain-enriched miRNAs, such as miR-222 and miR-212, have been reported to be involved in development of addiction by directly manipulating dendritic spine morphogenesis, synaptic remodeling, and the rewarding properties of drugs, drug-seeking behavior, and self-administration rates of alcohol [13, 15]. Identification of specific miRNAs involved in the addiction process is important to diagnosis. Emerging evidence suggests that brain and circulating miRNAs can be biomarkers of psychiatric disorders and addiction states [16, 17]. Previous studies demonstrated that miRNA expression is associated with several physical and mental health disorders [18, 19]. However, alterations of various

miRNAs levels in patients with methamphetamine abuse disorder are largely unclear. Therefore, identifying specific miRNAs is associated with methamphetamine abuse disorder and can develop as a novel diagnosis methods and therapeutic approach for patients with methamphetamine abuse disorder.

We evaluated expression of miR-222 and miR-212 in peripheral blood of patients with methamphetamine abuse disorder. Our results demonstrated that the expression levels of miR-222 significantly upregulated (12.9-fold change) in patients with methamphetamine abuse disorder as compared with healthy controls. However, expression levels of miR-212 insignificantly upregulated in patients with methamphetamine abuse disorder. In addition, the ROC analysis showed that altered levels of miR-222 provided a potential biomarker for methamphetamine abuse disorder in forensic and clinical applications.

A similar study by Du et al. reported that in the rats with escalated methamphetamine use, miR-127, miR-186, miR-222, and miR-24 were verified to be up-regulated, while miR-329 was down-regulated compared with controls [20]. Another study by Hollander et al. demonstrated that miR-212 and miR-132 were upregulated in the dorsal striatum in the extended access rats 24 h after the last cocaine self-administration session relative to the yoked, restricted access and cocaine-naïve control groups [21]. In recent years, evidence suggested that the blood expression of other several miRNAs is involved in methamphetamine and other drugs abuse disorders. A study by Gu et al. suggested that the serum expression of miR-9-3p was significantly increased in



patients with methamphetamine abuse disorder [22]. Zhao et al. reported that the plasma expression of miR-let-7e, miR-let-7d, miR-15b, and miR-181a significantly was decreased in patients with methamphetamine abuse disorder [23]. An another study by Zhang et al. suggested that the blood expression of miR-181a was significantly down-regulated in patients with chronic methamphetamine disorder [24]. In the present study, we demonstrated a significant upregulation in the blood levels of miR-222 in patients with methamphetamine abuse disorder compared to healthy controls. These studies may present the important role of various miRNAs in regulating methamphetamine addiction. However, underlying molecular mechanisms and other physiological and pathological role of various miRNAs in patients with methamphetamine abuse disorder remain unknown. Moreover, further studies reported that various miRNAs involved in methamphetamine addiction play a pivotal role in psychological and neurological disease by involving several signaling pathways, such as gonadotropin-releasing hormone (GnRH), mitogen-activated protein kinase (MAPK), cAMP Response Element-Binding Protein (CREB), G-protein, and Couple Receptor [24, 25]. Nevertheless, the exact roles of miR-222 and miR-212 in physiological and pathological processes in patients with methamphetamine abuse disorder require further investigation. In addition, longitudinal studies are required to identify the potential miRNAs biomarkers for diagnoses and prognosis of patients with methamphetamine abuse disorders.

### Limitations

There are several limitations of this study. This study could not explain the exact molecular mechanism related to miR-222 and miR-212 expression. It is better to monitor the expression of miR-222 and miR-212 during the treatment procedure. The sample size was small, and the study population was very concise. Thus, cautious interpretation is advised.

### Conclusion

In conclusion, the present study suggested that miR-222 may play an important role in the pathology of methamphetamine abuse disorder and can be used as a potential blood biomarker for diagnosing patients with methamphetamine abuse disorder. However, further studies are required to determine the exact role of miR-222 in genetic pathways or specific targets in patients with methamphetamine abuse disorder.

### Abbreviations

miR: MicroRNAs; LC: Liquid chromatography; MS: Mass spectrometry; GC: Gas chromatography; EDTA: Ethylene diamine tetra acetic acid; CT: Threshold

cycle; DEPC: Diethyl pyrocarbonate; ROC: Receiver operating characteristics; AUC: Area under curve; GnRH: Gonadotropin-releasing hormone; MAPK: Mitogen-activated protein kinase; CREB: CAMP Response Element-Binding Protein.

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### Authors' contributions

HS conceived the study. SF and HS designed the study. AT and ZA wrote the draft manuscript. SRM edited and revised the manuscript. All the authors approved the final version of the manuscript.

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### Availability of data and materials

All the data are provided within the manuscript.

### Declarations

#### Ethics approval and consent to participate

All patients and healthy controls signed a consent form as Declaration of Helsinki ethical standards, and approved by Ethics Committee of Islamic Azad university, Tabriz Branch (IR.IAU.TABRIZ.REC.1398.082).

#### Consent for publication

Written informed consent was provided by all participants for publication.

#### Competing interests

The authors declare that they have no competing interests.

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